Bud Hardiness and Deacclimation in Blueberry Cultivars With Varying Species Ancestry: Flowering Time May Not Be a Good Indicator of Deacclimation

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Abstract

Blueberry cultivars with varying percentages of species ancestry (*V. corymbosum* L., *V. angustifolium* Ait., *V. ashei* Reade, *V. darrowi* Camp) were assayed in mid-February to determine initial bud hardiness, and rates of deacclimation under constant temperature conditions. The LT_{50} (the temperature at which 50% lethality occurs) of detached shoots of field-grown plants of 'Weymouth', 'Bluecrop', 'Legacy', 'Ozarkblue', and 'Tifblue' were evaluated at Day 0 by controlled freezing in a glycol bath at temperatures from -1°C to -28°C, followed by visual evaluation after a 24h incubation at 23 °C. Similar shoots were deacclimated at a constant temperature of 20 °C and a new batch was evaluated daily for 6 days. Cultivars with any amount of southern germplasm (*V. ashei* or *V. darrowi*) were less hardy (LT_{50} = -20 to -21 °C) than northern highbush cultivars (LT_{50} = -24 °C) which are composed primarily of *V. corymbosum* with small percentages of *V. angustifolium*. Cultivars with greater amounts of southern germplasm ('Legacy', 'Ozarkblue', and 'Tifblue') started at less hardy levels, and deacclimated to a slightly less hardy level (LT_{50} = -12 to -14 °C) than did northern-adapted cultivars ('Weymouth' and 'Bluecrop') (LT_{50} = -15 °C). By Day 6, deacclimation appeared to plateau for all cultivars. Cultivar deacclimation was modeled using a log-linear regression model. Northern and southern cultivars differed in their regression parameters. 'Ozarkblue', an extremely late-flowering cultivar, would seem to be adaptable to northern climates, yet the data from this study suggest bud swell and flowering time may be poor measures of rates of deacclimation. Deacclimation under fluctuating field conditions is currently being evaluated.

INTRODUCTION

The development of more cold hardy and spring frost resistant cultivars is an important need to the blueberry industry in the United States (Moore, 1993). Over the past several years, the research group at the Beltsville Fruit Laboratory has focused on studying the genetics of mid-winter cold hardiness in the hope of using what is learned to aid in developing more cold hardy cultivars. In these studies, we have identified dehydrin genes and demonstrated that levels of these proteins are correlated with cold hardiness levels (Muthalif and Rowland, 1994) and have made progress toward mapping QTLs (quantitative trait loci) controlling mid-winter cold hardiness (Rowland et al., 1999) in blueberry.

More recently, we have initiated a study to address the need to develop cultivars more resistant to spring frosts. For this, we have focused on deacclimation (the loss of cold hardiness with exposure to warm temperatures), as opposed to cold acclimation. For cultivars that exhibit early spring frost damage, several possibilities exist with respect to cause: 1) the cultivar may lack sufficient mid-winter cold hardiness, 2) the cultivar may be sufficiently cold hardy mid-winter but may deacclimate too quickly in the spring, or 3) a combination of both, the cultivar may lack sufficient cold hardiness and deacclimate quickly. In an effort to better understand deacclimation in blueberry, we have measured and developed models to describe deacclimation in several blueberry cultivars with different germplasm compositions and different phenological field responses. We then examined these models to determine their predictive value.

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MATERIALS AND METHODS

Detached shoots of blueberry cultivars with varying percentages of species ancestry *(V. corymbosum* L., *V. angustifolium* Ait., *V. ashei* Reade, *V. darrowi* Camp) were assayed in mid-February in 2000 and 2001 to determine bud hardiness, and to evaluate rates of deacclimation under constant temperature conditions. The germplasm composition of the cultivars used, 'Weymouth', 'Bluecrop', 'Legacy', 'Ozarkblue', and 'Tifblue', are listed in Table 1. Germplasm composition was determined from previously published sources (Ehlenfeldt, 1994; Hancock and Siefker, 1982; Clark, et al., 1996). Shoots of 'Weymouth', 'Bluecrop', 'Legacy', and 'Ozarkblue' were collected from a commercial farm in Hammonton, New Jersey. Shoots of 'Tifblue' were collected from a field plot at the Henry A. Wallace Agricultural Research Station at Beltsville, Maryland. The LT_{50} values of shoots of were first measured at Day 0 by controlled freezing in a glycol bath (Forma Scientific, Marietta, Ohio) as previously described by Arora et al. (2000). For the freeze-thaw test, 3 shoots with at least 3 attached buds per treatment temperature (-1°C to -28°C, at 2 °C increments) were frozen, followed by visual evaluation after a 24h incubation at 23°C to determine percent damage. In 2000, similar sets of shoots were deacclimated at a constant temperature of 20°C for increasing 3-day intervals (i.e, one set deacclimated for 3 days, the next set deacclimated for 6 days, etc) up to 15 days, then evaluated similarly. In 2001, shoots were deacclimated for increasing 1-day intervals up to 6 days. Controls in both years consisted of similarly handled shoots with no exposure to glycol bath freezing regimes. LT_{50} values were estimated using Proc Probit in SAS version 8.2 by fitting percent damage versus temperature to a logistic (i.e. sigmoidal) regression model. The single LT_{50} estimates obtained at each day from these models were subsequently used to model the relationship between LT_{50} and days. For each year and genotype, followed by each genotype (combining years), a log-linear model was fit to the LT_{50} versus day data.

Data on flowering times were collected from a cultivar plot located at the Philip E. Marucci Center for Blueberry and Cranberry Research and Extension at Rutgers University, Chatsworth, New Jersey from 1999 to 2002. The plot was surveyed once a week, usually on Tuesday, from the initiation of flowering of the earliest-blooming cultivar until the end of fruiting of the latest-ripening cultivar. The values represent a composite estimate across five representative plants of each cultivar. "Start of flowering" dates represent the week-long interval during which flowering initiated. The 50% bloom time was determined by interpolation from bracketing values.

RESULTS AND DISCUSSION

In 2000, it was observed that deacclimation occurred quickly at 20°C, and appeared to plateau for all cultivars by day 6. Therefore, in 2001 deacclimation assessments focused on this smaller, critical, 6-day period. During this time frame, 'Legacy', 'Ozarkblue', and 'Tifblue' started at a less hardy level (LT_{50} = -20 to -21 °C) and deacclimated to a slightly less hardy level (LT_{50} = -12 to-14°C) than did the cultivars, 'Weymouth' and 'Bluecrop'. 'Weymouth' and 'Bluecrop' started at an LT_{50} of -24 to -25°C and deacclimated to an LT_{50} of -15 °C. (Figure 1). By the end of this period all cultivars had reached a comparable plateau level.

The relationship between LT_{50} and days of deacclimation for all years and genotypes were initially modeled individually; however, after preliminary analysis, that showed no statistically significant differences between years, years were combined and reanalyzed over the critical interval from Day 0 to Day 6. Data from 0, 3, and 6 days from 2000 were combined with data from 0-6 days from 2001 and a single log-linear regression model developed for each genotype (Figure 2). 'Legacy' and 'Ozarkblue' could be described by identical models. 'Bluecrop' and 'Weymouth' models were similar to each other, but not identical, and the 'Tifblue' model differed from all of the others (Table 2). In a comparison of initial LT_{50} values (predicted from the models), significant differences were observed between the two groups of cultivars, one consisting of 'Weymouth' and 'Bluecrop', and the other consisting of 'Ozarkblue', 'Legacy', and

‘Tifblue’ (Table 3). The group consisting of ‘Bluecrop’ and ‘Weymouth’ was more cold hardy than that of the cultivars with southern germplasm.

Rates of deacclimation derived from the log-linear regression models for all cultivars were compared across days 0 to 3, the period during which the largest decreases in LT_{50} occurred. The only significant difference observed was between ‘Legacy’/ ‘Ozarkblue’ and ‘Tifblue’ on Day 3. ‘Tifblue’ had a higher rate of deacclimation than ‘Legacy’/’Ozarkblue’.

Cold hardiness levels during deacclimation at any given point in time is a function of both initial LT_{50} and the rate of deacclimation. Hence, a meaningful way of evaluating LT_{50} values might be to ask how long it takes each genotype to reach a selected LT_{50} value (such as -15 °C, when deacclimation rates seem to slow down). From the models, ‘Weymouth’ took 4.25 days to reach an LT_{50} of -15°C, ‘Bluecrop’, 4.65 days; ‘Legacy’/ ‘Ozarkblue’, 2.00 days, and ‘Tifblue’, 1.45 days.

With regard to germplasm composition, cultivars with any amount of southern germplasm from *V. ashei* or *V. darrowi* (‘Legacy’, ‘Ozarkblue’, and ‘Tifblue’) were less hardy initially (Day 0) than northern highbush cultivars which are composed primarily of *V. corymbosum* with small percentages of *V. angustifolium* (‘Weymouth’ and ‘Bluecrop’). In addition, cultivars with greater amounts of southern germplasm deacclimated during the 6-day period to a less cold hardy level than did the northern-adapted cultivars.

An examination of bloom initiation and 50% bloom dates (Table 4) with respect to deacclimation values is informative. ‘Weymouth’, an early-ripening cultivar flowers early and reaches 50% bloom the most rapidly of the four highbush-type cultivars. ‘Bluecrop’ and ‘Legacy’ have similar initiation and 50% bloom dates. and hence they *might* be expected to behave similarly with respect to deacclimation. ‘Ozarkblue’ is late to begin flowering, and late to reach 50% bloom, and because of this, might be expected to be less susceptible to frosts and more adaptable to northern climates. The deacclimation data from this study showed that ‘Weymouth’ and ‘Bluecrop’ deacclimated similarly, and as expected since they are northern highbush cultivars with similar genetic compositions. ‘Legacy’ flowers similarly to ‘Bluecrop’, but because of its southern germplasm, it deacclimated from a higher starting point, deacclimated to a less cold-hardy level by 6 days, and as a result reached an LT_{50} of -15°C 2.65 days more quickly than ‘Bluecrop’. Although ‘Ozarkblue’ flowers late, it deacclimated identically to ‘Legacy’ and similarly to ‘Tifblue’. Initial cold-hardiness levels and their decline during deacclimation are strongly reflective of the percentages of non-*V. corymbosum* germplasm in cultivar ancestries (Table 1). Hence, the results suggest that field observations of bud swell (or lack of it) and flowering time may be poor measures of deacclimation. In the end, germplasm composition and ancestry may be most critical in predicting deacclimation behavior.

It would be unfair not to mention some preliminary results from another ongoing study. Currently, deacclimation under fluctuating field conditions is being evaluated. The same cultivars (plus others) were evaluated weekly under field conditions in 2002 to determine whether the types and rates of deacclimation observed in the continuous-temperature deacclimation experiments would be comparable to those seen under field conditions. Although no statistical analyses have yet been done, simple graphing of the results suggests that ‘Ozarkblue’, under field conditions, did not behave appreciably different from ‘Weymouth’ and ‘Bluecrop’. ‘Legacy’, however, had a lesser initial cold hardiness level (LT_{50} = -19°C vs. <-25 °C for the other cultivars), and exhibited a period of rapid deacclimation and reached a plateau at approximately -14 °C for 3-4 weeks, before finally completing its deacclimation. These studies, in some aspects, differ from the steady-state acclimation results in our completed study and hint at other factors that need to be considered. The slow budbreak of cultivars like ‘Ozarkblue’ may indicate that “protective” cold-hardiness levels are maintained under actual field conditions.

CONCLUSION

The results of this study enhance our understanding of cold hardiness and deacclimation, but new answers beget new questions. It seems clear that genetic composition is a critical factor in making predictions of cold-hardiness in mid-winter and during deacclimation. This study examined deacclimation under steady-state conditions. This gives excellent information on the deacclimation process with most variables controlled. Studies underway have hinted that deacclimation under field conditions may introduce new considerations to our ultimate understanding of deacclimation. It seems clear that field deacclimation is neither uniform nor continuous. Starting cold-hardiness levels may be critical, and cultivars may respond to warming temperatures in very different ways. Further field studies will deepen our understanding of this process.

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Table 1. Germplasm composition of cultivars evaluated for cold hardiness and deacclimation rates.

Cultivar	*V. corymbosum* (%)	*V. angustifolium* (%)	*V. darrowi* (%)	*V. ashei* (%)
Weymouth	82.5	12.6	-	-
Bluecrop	93.7	6.3	-	-
Legacy	73.4	1.6	25.0	-
Ozarkblue	77.4	3.9	11.3	7.0
Tifblue	-	-	-	100.0

Table 2. Model parameters for combined-year log-linear regression model of deacclimation data.

Cultivar	LT_{50} model a + [b * log (day + c)]
Weymouth	-29.43 + [7.85 * log (day + 2.03)]
Bluecrop	-29.43 + [7.85 * log (day + 1.66)]
Legacy	-22.94 + [6.13 * log (day + 1.66)]
Ozarkblue	-22.94 + [6.13 * log (day + 1.66)]
Tifblue	-22.94 + [7.04 * log (day + 1.66)]

Table 3. Day 0 - LT50 estimates and confidence limits from combined-year log-linear regression models of deacclimation.

Cultivar	Estimate ± confidence limit	df	t-value	Pr > t
Weymouth	-23.88 ± 1.90	47	-25.71	< 0.0001
Bluecrop	-25.46 ± 1.88	47	-27.31	< 0.0001
Legacy	-19.83 ± 1.23	47	-32.50	< 0.0001
Ozarkblue	-19.83 ± 1.23	47	-32.50	< 0.0001
Tifblue	-19.37 ± 1.72	47	-22.75	< 0.0001

Table 4. Average flowering times of cultivars in Burlington County, New Jersey, 1999-2002.

Cultivar	Start of flowering	50% bloom
Weymouth	4/15 - 4/22	4/22
Bluecrop	4/19 - 4/26	5/2
Legacy	4/19 - 4/26	4/29
Ozarkblue	4/23 - 4/30	5/9
Tifblue	n.a.	n.a.

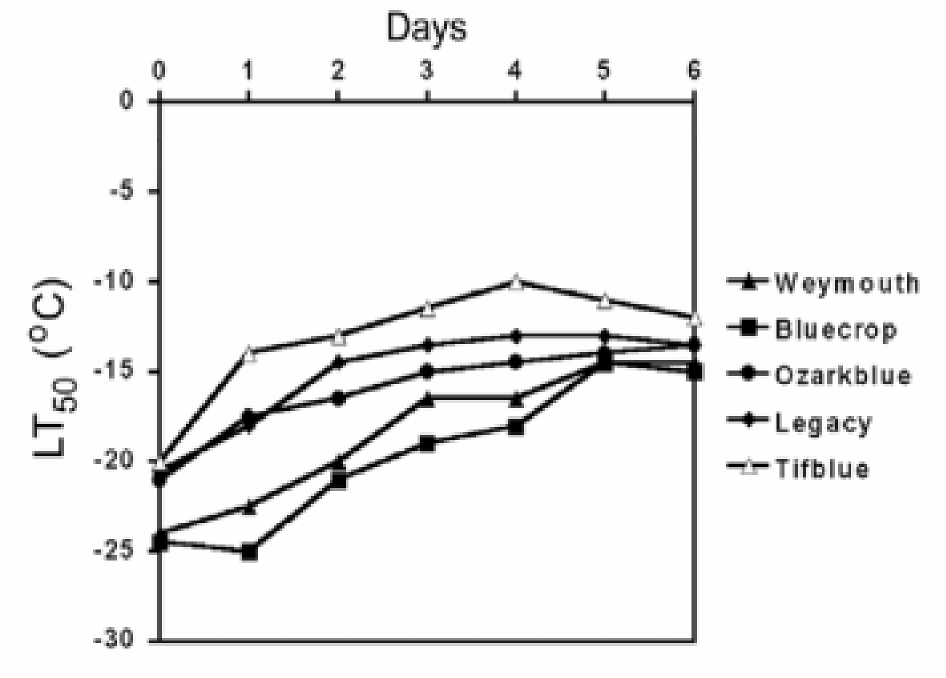

Fig. 1. Deacclimation of blueberry cultivars from Day 0 to Day 6 in 2001.

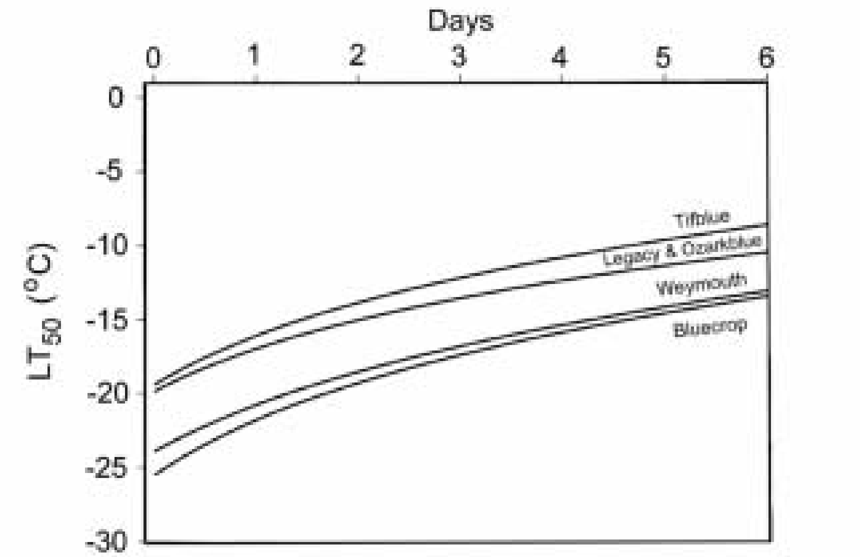

Fig. 2. Log-linear regression plots of blueberry deacclimation across 2000 and 2001.